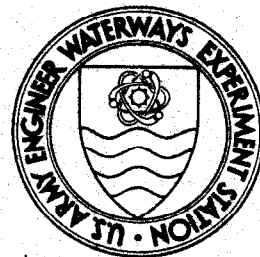


# DREDGED MATERIAL RESEARCH PROGRAM



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## A BIOASSAY DILUTION TECHNIQUE TO ASSESS THE SIGNIFICANCE OF DREDGED MATERIAL DISPOSAL

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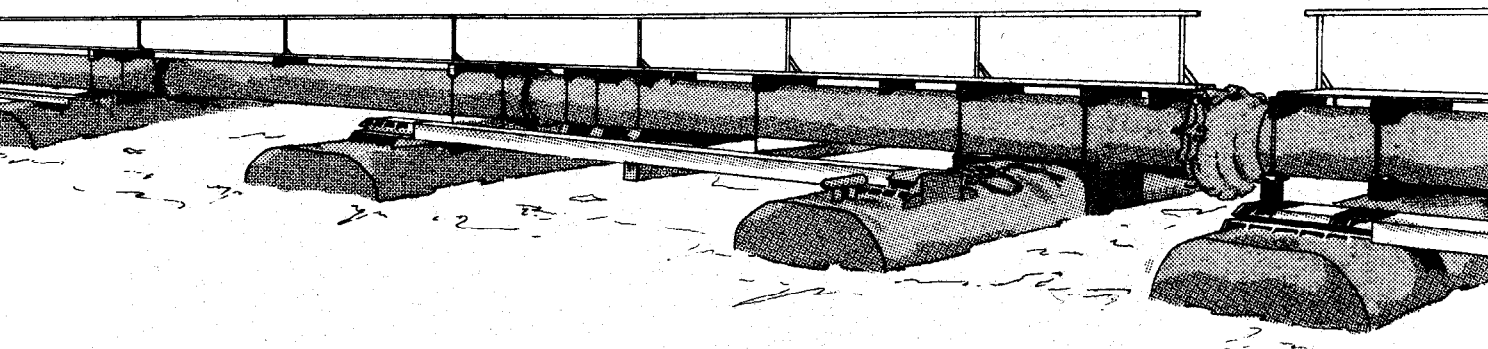
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specified in conventional bioassays. The results demonstrate that stimulatory or inhibitory materials do not have a significant effect on an algal population when the rate of dilution at an open-water site is considered.

## Preface

This paper was originally published in the work entitled "Bio-stimulation and Nutrient Assessment" as a part of the Biostimulation and Nutrient Assessment Workshop held at Utah State University, Logan, Utah, 10-12 September 1975.

Section 404(b) of Public Law 92-500 stated that guidelines would be developed to issue permits for the discharge of dredged or fill material at specified sites. As a consequence, guidelines were published in the 5 September 1975 Federal Register to evaluate proposed dredged and fill material operations. The guidelines referred to several technical procedures, including bioassays, that could be used in the evaluation process. However, conventional bioassay procedures must be modified to reflect the rapidly changing concentration-time conditions at a dynamic location, such as a disposal site, if the results are to be of value in assessing the potential effects of dredged material disposal. This report presents initial bioassay results based on a modified bioassay procedure.

This project was not a funded Dredged Material Research Program (DMRP) work unit. The objective of the workshop presentation, however, was to suggest procedures that could be used to assess the environmental impact of open-water disposal of dredged material, which falls within the scope of DMRP Task 1E, Pollution Status of Dredged Material. Information from that task as well as research performed by the author at the University of Texas-Dallas was used as the basis for the technique reported herein.

This report was prepared by Dr. R. H. Plumb, Jr., of the Environmental Impacts and Criteria Development Project of the DMRP and the Ecosystem Modeling Branch of the Environmental Effects Laboratory (EEL). Dr. J. W. Keeley, Special Assistant for Program Development, EEL, Dr. R. M. Engler, Manager, Environmental Impacts and Criteria Development Project, and Dr. J. Harrison, Chief, EEL, also assisted in the preparation of this report.

Directors of WES during preparation of this report were COL G. H. Hilt, CE, and COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

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A BIOASSAY DILUTION TECHNIQUE TO ASSESS THE SIGNIFICANCE  
OF DREDGED MATERIAL DISPOSAL

Introduction

1. One of the potential problems at an open-water dredged material disposal site is that the nutrients associated with dredged sediment and its interstitial water may stimulate the growth of algae at the site. The basis for this concern is the relatively high levels of nitrogen and phosphorus that have been reported in the sediment. Unfortunately, the reported concentrations are generally bulk sediment analysis results that fail to consider the chemical form or availability of the chemical in question. However, there is a growing awareness of this problem, and investigations are in progress to evaluate an Elutriate Test (Lee and Plumb, 1974), a short-term leaching test, as a measure of sediments potential to release chemicals to surrounding water during disposal operations. Lee and Plumb (1974) suggested that bioassays are necessary in the Elutriate Test evaluation since the potential problems of algal stimulation are associated with nutrient availability and nutritional status of the algae in much the same way that chemical release is affected by chemical form and availability rather than total sediment concentration.

2. An appreciation of conditions at an open-water site makes it evident that the conventional bioassay methodology is not applicable to a discrete discharge operation. Prior to the discharge of dredged material, the site can be considered to have a uniform distribution of algae. Immediately after the disposal of dredged material, a portion of the population will be exposed to some high initial concentration. With the passage of time, the high initial concentration will be diluted by mixing with surrounding waters. Thus, the initially exposed algae will have been in contact with a high average concentration  $C_0$  for a time interval  $t_1 - t_0$  and a lower average concentration  $C_1$  for a time interval  $t_2 - t_1$ . This step is not simply dilution, however, since the

dilution water also contains algae that will be exposed to an average concentration  $C_1$  for the time interval  $t_2-t_1$ . As the process continues, increasing numbers of algae will be exposed to the discharge but at decreasing concentrations.

3. It becomes apparent that the constant concentration and the exposure times of 96 hr to 2 weeks used in a standard bioassay are unrealistic and that results from such a test will only provide information on the availability of nutrients but not on the significance of the nutrients because the test conditions do not approximate those at a disposal site. Since open-water disposal is an intermittent event that produces some high initial concentration that is rapidly diluted with the passage of time, algal assays to assess the significance of open-water disposal must incorporate a dilution technique as a reasonable approximation to actual conditions.

#### Approach

4. The Elutriate Test (Lee and Plumb, 1974) is one of several procedures specified in Section 404(b) of the 1972 Federal Water Pollution Control Act to evaluate the ecological impact of proposed dredged material disposal operations. The test specifies a ratio of one volume sediment to four volumes dredging site water as an approximation of the slurry discharged during hydraulic dredging. A conventional bioassay could be run on the filtrate from the Elutriate Test, the standard elutriate, but this would only indicate bioavailability of materials in the elutriate and not significance of the discharge.

5. As indicated in Figure 1, the concentration of a bioavailable constituent in the standard elutriate would represent a maximum instantaneous concentration resulting from the discharge of dredged material that would be expected to decrease with time due to the combined effects of mixing, advection, and settling. Since the time required for dilution is short compared with the exposure time used in the conventional bioassay evaluation, the significance of the discharge would be less than the bioassay results would indicate. However, the use of a

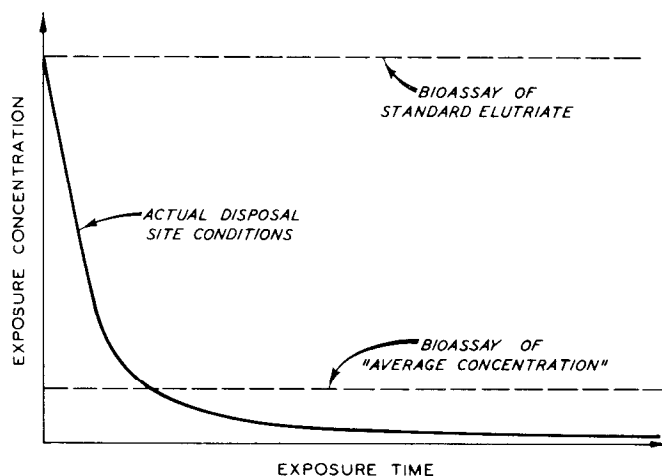


Figure 1. Schematic comparison of actual disposal site conditions with bioassay results of the standard elutriate and an average elutriate concentration

calculated average concentration would not necessarily provide a better estimate of the significance of a dredged material discharge since possible shock effects of the high initial concentration would not be considered (Figure 1).

6. The bioassay procedure discussed in this paper incorporates a serial dilution technique as a reasonable approximation of the changing concentration-exposure time relationship that would be expected following the discharge of dredged material at an open-water site. The implementation of the approach required the development of an expression for the expected rate of dilution. An empirical relationship between apparent dilution and time was obtained based on dye diffusion data collected by Carter and Okubo (1965). These studies were conducted over a 3-yr period with different types of dyes at different initial concentrations, in different water areas along the Florida coast, and at different times of the year. The Carter and Okubo (1965) data were transformed on the assumptions that the concentration at any time  $t$  was proportioned to the initial mass of dye released and that the concentration would decrease with time according to the equation

$$C = Me^{-kt} \quad (1)$$

where

C = concentration at time t ,  $\mu\text{g}/\ell$

M = mass of dye released,  $\mu\text{g}$

k = diffusion coefficient,  $-\ln(\ell)/\text{hr}$

t = time since release, hr

7. The calculated diffusion coefficient k varies with time, but the transformed diffusion data could be described by the following equation

$$\log K = -0.94 (\log t) + 1.26 \quad (2)$$

with a correlation coefficient of 0.99 between  $\log K$  and  $\log t$  for 78 observations.

8. Equations 1 and 2 were then used to prepare a dilution curve from which the time intervals necessary for successive tenfold dilutions to occur were obtained (Figure 2). Equation 1 was used to calculate the

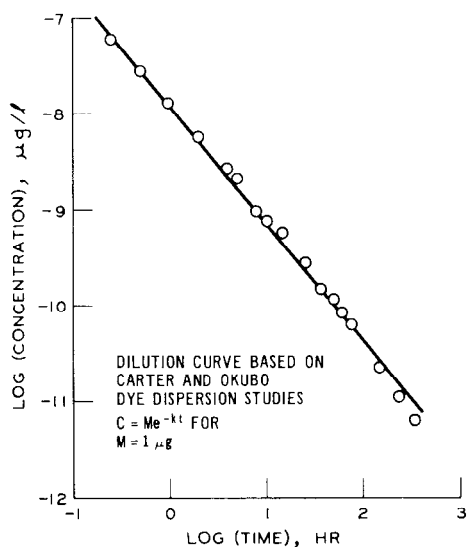


Figure 2. Concentration-time curve based on Carter and Okubo dye dispersion studies and a unit mass of 1  $\mu\text{g}$

expected concentration at 15 min. This value was selected as the "initial concentration" at an open-water disposal site because a discrete discharge would be expected to last approximately 30 min (Keeley, 1975). The necessary time intervals for successive tenfold dilutions to occur were then calculated to be 2, 15, 72, and 336 hr. That is, the concentration at 2 hr would be expected to be one-tenth of the 15-min concentration; the concentration at 15 hr would be expected to be one one-hundredth of the 15-min concentration, etc. In order to approximate average concentrations during

each time interval more closely, the times selected for dilution of the laboratory cultures were 1, 4, 22, and 96 hr. This follows from the fact that a dilution at the end of the calculated time interval would

overestimate the exposure concentration. Also, the times selected for dilution of the laboratory cultures were not the midpoint of the time (i.e., 1 hr is not the midpoint of the 15-min, 2-hr interval) because the dilution curve is a nonlinear function.

9. As a further approximation of open-water disposal, the water used for sequential dilution also contained algae. This follows from the fact that water at the disposal site not immediately affected by a discharge contains algae that will be influenced as a consequence of mixing and diffusion. However, these algae will be exposed to a lower concentration of the discharge for a different period of time.

### Procedures

10. Sediment samples and site water were collected from several locations in the Mobile, Alabama, Ship Channel in March 1975. This material was used to prepare standard elutriates as described by Keeley and Engler (1974). One volume of sediment was added to four volumes of site water and was shaken for 30 min. After a 1-hr settling period, the standard elutriate (filtrate) was obtained by centrifugation and filtration through a 0.45- $\mu$  membrane filter. This solution was bioassayed using *Dunaliella tertiolecta* as the test organism and filtered Mobile Harbor water as dilution water.

11. The experimental design consisted of duplicate flasks containing 100 ml of 100-percent standard elutriate (flasks A-1 and A-2) and eight flasks containing 90 ml of 0.45- $\mu$  filtered site water (flasks B-1, B-2, C-1, C-2, D-1, D-2, E-1, and E-2). In addition, controls consisting of 100 ml of site water and 100 ml of Provisional Algal Assay Procedure (PAAP) media (U. S. EPA, 1969) in site water were prepared in duplicate. At the beginning of the experiment, each flask was inoculated with  $2 \times 10^6$  cells per 100-ml solution.

12. One hour after the experiment began, 10 ml of culture A (100-percent standard elutriate) was added to flask B to simulate a tenfold dilution. Three hours later (4 hr after the experiment began), 10 ml of culture B was added to culture C. Sequential tenfold dilutions from culture C to culture D and culture D to culture E were performed

at 22 and 96 hr, respectively. The dilution times were selected based on an analysis of dye diffusion data discussed earlier and were used as the best available estimate of what is likely to happen at an open-water disposal site.

13. Two types of algal cultures were used in the initial experiments described in this paper. One was *Dunaliella tertiolecta* grown in PAAP media and 8 ‰ artificial seawater and the second was *Selenastrum capricornutum* grown in PAAP media. The PAAP media were prepared as needed by dilution of stock nutrient solutions (U. S. EPA, 1969).

### Results

14. Results of the *D. tertiolecta* bioassay are presented in Table 1. For the PAAP, site water, and 100-percent elutriate cultures, the reported cell counts were observed at the specified time after the start of the experiment. The results for culture labeled "Dilutant" were taken from different cultures depending on the elapsed time after the start of the experiment. The 24- and the 48-hr observations were taken from culture D in the sequential dilution series and the 96-, 192-, and 432-hr observations were taken from culture E in the same series. The reason for choosing these cultures is that, based on the dye diffusion studies of Carter and Okubo (1965), these cultures represent the extent of dilution to be expected at an open-water disposal site at the time the cultures were sampled.

15. PAAP media cultures peaked at 1.5 million cells/ml between four and eight days and decreased to 800,000 cells/ml at day 18. The site water population reached approximately 250,000 cells/ml on day 4 and decreased slightly to 200,000 cells/ml at day 18. The algal populations in the 100-percent elutriate cultures were similar to the population in the site water cultures for the first eight days. However, by the 18th day, a significant reduction from 200,000 to 10,000 cells/ml had occurred.

16. Another qualitative difference between the 100-percent elutriate cultures and the control cultures was apparent during the

counting since *D. tertiolecta* is a motile organism. Samples were not fixed and the test organisms were seen to be motile in the PAAP and site water cultures but not in the elutriate cultures. This would suggest that the populations in the elutriate cultures were under stress even though the population increased during the first eight days and that the stress finally resulted in a lower population on day 18. By comparison, the *D. tertiolecta* population in the dilution sequence maintained a motility similar to the site water cultures. Thus, even though the population was exposed to a toxic or inhibitory condition, there were no qualitative indications of stress and the final cell count on day 18 would suggest that no significant effect on the algal population would result from the open-water disposal of Mobile Harbor sediments.

17. Observed cell counts in all cultures after 432 hr are presented in Table 2. Maximum cell counts of 800,000 cells/ml were present in the PAAP growth medium cultures. The lowest cell counts, 10,000 cells/ml, were observed in the 100-percent elutriate cultures (A), and the next lowest algal population occurred in the flasks containing 10-percent elutriate culture (B) for all but 1 hr of the experiment. These results are in agreement with unpublished results of Shuba (1975), who observed that 25-, 50-, 75-, and 100-percent solutions of Mobile Harbor elutriates were toxic to *D. tertiolecta*. Results from the 1-percent elutriate cultures (C), 0.1-percent elutriate cultures (D), and 0.01-percent elutriate cultures (E) were essentially the same as the site water cultures (SW).

18. The importance of considering the concentration-exposure time regime at an open-water disposal site in order to assess the significance of discrete discharges was further demonstrated by running a dilution bioassay of PAAP media. The experimental setup was essentially the same as described above, except that complete PAAP media were used in place of the standard elutriate; PAAP media without phosphate addition (PAAP-P) were used as dilution water; and *Selenastrum capricornutum* was used as the test organism. The experimental design would be similar to discharging phosphorus-rich waste into a phosphorus-limited environment.

19. Results of the dilution bioassay, presented in Table 3, demonstrated that the highest cell counts were observed in complete PAAP media (A and P), as would be expected. These values were approximately 2,000,000 cells/ml after 123 hr and 4,000,000 cells/ml after 291 hr. The 10-percent PAAP media cultures (B) had cell counts approximately one order of magnitude lower than the complete PAAP media cultures. Cell counts in the remaining cultures of the PAAP dilution sequence (C,D,E) were not different from the PAAP-P media used as a control (H).

### Discussion

20. All chemical constituents present in natural water systems are not equally available to aquatic organisms and one purpose of conducting bioassays is to determine what fraction of the total chemical concentration is available. However, as pointed out by Brown (1973), the concentration capable of producing some selected response is a function of the duration and nature of the exposure. This fact becomes apparent after examining any published list of toxicity data, such as that reported by McKee and Wolf (1963). Therefore, time of exposure is an important factor that must be considered in assessing the significance of a discrete discharge such as dredged material or an industrial spill. This is particularly true for those situations where the resultant concentrations persist for time periods that are short compared with those specified in bioassay procedures used to develop water-quality criteria.

21. Brown (1973) has stated that the duration of a toxicity test should not be selected on convenience but should have some rational basis. Results presented in this paper demonstrate the importance of properly considering exposure time in order to assess the significance of a discrete discharge such as dredged material disposal in open water. Substances shown by conventional bioassay procedures to be inhibitory (Mobile Harbor elutriate) or stimulatory (PAAP media) did not have a significant effect on the test population when the rate of dilution was simulated. Although the difference in results between conventional

bioassay procedures and the dilution bioassay procedure can only be resolved with the collection of sufficient field data, the fact that disturbances due to dredging activities have become undetectable within 2 hr of disposal termination in some cases (May, 1973) is sufficient justification to consider the rate of dilution at a disposal site in order to assess the potential effects of open-water disposal.

22. The dilution equations developed in this work may not be strictly applicable to dredging activities because they are based on dye diffusion studies rather than suspended solids dispersion. However, the rate of dilution used in the experiments can probably be considered conservative because suspended solids would be removed from the water column faster than a soluble dye. Also, Carter and Okubo (1965) reported the maximum concentration remaining in the dye cloud at the time of sampling and not the change in concentration at a single point in space, which would further tend to make the rate of dilution that was used conservative.

23. It should be pointed out that the development of a single dilution equation in this study does not imply that the equation is universally applicable. The Carter and Okubo (1965) data set was utilized because they had sufficient data to define a dilution curve and because the data covered a time interval similar to that used in algal bioassays. As more data become available on open-water disposal and better equations are developed to describe the rate of dilution, the bioassay procedure can be modified, but this will only require changing the time interval between successive dilution steps. In addition, if it is desirable to study the possible effects of a discharge in more detail and a dilution curve is available, dilution factors of two or five can easily be substituted for the factor of ten used in this study. It is also apparent that the conventional bioassay is only a special case of a dilution bioassay (initial concentration is specified with a dilution rate of zero).

24. One of the main objectives of bioassay procedures is the proper assessment of the hazard associated with any given pollutant (Brown, 1973). Because the hazard associated with a pollutant will be

a function of the exposure concentration and the exposure time, it is suggested that the described dilution procedure would provide more representative estimates of the possible significance of open-water disposal of dredged material since the method provides the flexibility to consider site specific hydrodynamic factors that will affect exposure time and concentration. An estimated 290 million cu m of sediment is annually dredged from the Nation's waterways (Boyd et al., 1972), and some type of regulatory decision must be made to place this material on land or at some other location in the waterway. It is felt that the dilution bioassay procedure will provide a more rational basis for deciding between the potential effects of open-water disposal and the higher costs of on-land disposal.

### Summary

25. A bioassay method has been proposed to assess the practice of open-water disposal of dredged material. The proposed method is based on nonlinear diffusion data observed in the field and approximates the changing concentration-time relationships that will exist at an open-water disposal site. A dilution bioassay is necessary because a conventional bioassay will only indicate the bioavailability of constituents associated with dredged material and not the significance of the discharge due to the fact that conventional bioassays are run for longer periods of time compared with the duration of dredged material perturbations. Results demonstrated that inhibitory and stimulatory additions would not have a significant effect on algae when the rate of dilution at an open-water site is considered. It is anticipated that the proposed procedure could provide necessary information in determining the appropriate method of dredged material disposal.

### Acknowledgement

26. The tests described and the resulting data presented herein, unless otherwise noted, were obtained from research conducted under the

Dredged Material Research Program of the United States Army Corps of Engineers. Permission was granted by the Chief of Engineers to publish this information. Support during part of this investigation was obtained from the Institute for Environmental Sciences, University of Texas at Dallas, Richardson, Texas.

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Table 1  
Dilution Bioassay Results of Mobile Harbor Sediment  
Elutriates Using *Dunaliella tertiolecta*

Culture	Sample No.	Test Duration--Cell Counts				
		24 hr	48 hr	96 hr	192 hr	432 hr
PAAP	1	50	225	1526	1475	748
	2	60	195	1428	1308	848
Site water	1	90	108	218	--	205
	2	50	120	278	220	198
100-percent elutriate	1	10	115	248	240	7.5
	2	30	95	228	172	12.5
"Dilutant"	1	60	172	--	190	240
	2	50	110	245	272	198

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Note: All cell counts are the average of duplicate cell counts of duplicate subsamples from each culture. The initial inoculum was  $2 \times 10^6$  cells/100 ml. Cell counts are expressed as  $10^3$  cells/ml.

Table 2

Dilution Bioassay Results of Mobile Harbor Sediment Elutriates  
After 432 Hours Using *Dunaliella tertiolecta*

<u>Experimental Conditions</u>	<u>Culture</u>	<u>Cell Counts</u>
100-percent elutriate from t = 0 to t = 432	A-1 A-2	7 12
Site water from t = 0 to t = 1 and 10-percent elutriate from t = 1 to t = 432	B-1 B-2	22 60
Site water from t = 0 to t = 4 and 1-percent elutriate from t = 4 to t = 432	C-1 C-2	215 205
Site water from t = 0 to t = 22 and 0.1-percent elutriate from t = 22 to t = 432	D-1 D-2	202 232
Site water from t = 0 to t = 96 and 0.01-percent elutriate from t = 96 to t = 432	E-1 E-2	240 192
Site water from t = 0 to t = 432	SW-1 SW-2	205 198
Site water plus PAAP from t = 0 to t = 432	P-1 P-2	748 848

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Note: Cell counts are expressed as  $10^3$  organisms/ml. All counts are the average of duplicate counts of duplicate subsamples from each culture. The initial inoculum in each culture was  $2 \times 10^6$  cells/100 ml. All times are given in hours. Elutriate percentages are based on percentages to be expected following successive tenfold dilutions.

Table 3

Results of a Dilution Bioassay of PAAP Media Using*Selenastrum capricornutum*

<u>Experimental Conditions</u>	<u>Culture</u>	<u>Test Duration Cell Counts</u>	
		<u>123 hr</u>	<u>291 hr</u>
PAAP media from t = 0 to t = 291	A	210	400
PAAP-P media from t = 0 to t = 1 and 10-percent PAAP media from t = 1 to t = 291	B	30	41
PAAP-P media from t = 0 to t = 4 and 1-percent PAAP media from t = 4 to t = 291	C	6.0	3.5
PAAP-P media from t = 0 to t = 22 and 0.1- percent PAAP media from t = 22 to t = 291	D	2.0	--
PAAP-P media from t = 0 to t = 100 and 0.01-percent PAAP media from t = 100 to t = 291	E	3.0	3.5
PAAP media from t = 0 to t = 291	P	240	400
PAAP-P media from t = 0 to t = 291	H	2.0	3.5

Note: Cell counts are expressed as  $10^4$  organisms/ml. All times are given in hours. PAAP media percentages are based on percentages to be expected following successive tenfold dilutions.

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